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PAIRED ION CHROMATOGRAPHY OF CERTAIN MONOSACCHARIDES.(U)
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A paired ion dye-impregnated reversed phase chromatographic column is shown to give well defined chromatographic zones for monosaccharides as their borate complexes, permitting determination of sub-microgram levels of fructose, xylose, and ribose.

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TECHNICAL REPORT NO. 5

"Paired Ion Chromatography of Certain Monosaccharides"

by

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submitted to

Analytical Chemistry

University of Arizona
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Paired ion Chromatography of Certain Monosaccharides

Sir:

Several chromatographic methods (1,2,3) have been reported for separation of sugars but this still remains a formidable task for determination of trace levels. The packings which have been most successful in separating sugars are those with amine groups bound to inert silica or synthetic supports such as u-Bondapak carbohydrate (Waters Assoc.), Lichrosorb-NH_2 (Merck) and Aminex resins (Bio-Rad Lab) (4). These methods involve elution with water/acetonitrile system or water at 80-85°C.

In the course of our study in reverse phase ion-pair HPLC (5) we found that sugars such as fructose, can be ionized on column and paired with a chromophore to effect improved separation and detection.

One of the interesting aspects of this separation is that the non-ionic species react on the column to form suitable species and separated. Generally the analyte is subject to chemical pre-treatment before separation. This is the first report of an on-column reaction in reverse phase HPLC.

Experimental

A modular chromatographic unit consisting of an Altex Pump (Model 100A), a variable-wave length uv-vis detector (Schoeffel Instruments No. 770) and a Fisher Recordall Series 500 strip chart recorder was used. Absorbance measurements were made at 651nm. A 25cm Zorbax ODS column, described by the manufacturer as having 15% carbon loading, was employed.

The mobile phase used was methanol-(0.1M aqueous borate buffer PH 7.5) in 2:98 V/V% containing 1×10^{-4} M methylene blue (as chloride).

Before the chromatographic separations were undertaken, the column was conditioned by passing sufficient mobile phase to "saturate" it with methylene blue (6). The flow rate used for both column saturation and subsequent chromatography was 1.0ml per min. (1% W/V) solutions of the fructose, xylose and ribose (separately) in the mobile phase were added through 10µl loop sampling valve (rotary valve injector SP-419-0410). As may be seen from the data, reasonable separation of the three sugars tested was obtained at sub-microgram detection limits ($\sim 10^{-7}$ g).

Table 1. Capacity Factors of Monosaccharides

Sugars	k′
Fructose	3.5
Ribose	2.9
Xylose	1.2

Mobile phase: 0.1M Borate Buffer pH7.5
Methylene blue 10^{-4}M
2% MeOH/H_2O V/V

Flow rate: 1.0ml per min.

Sample: 10µl 1.0% wt/V Solutions

The use of borate complex formation to produce anionic sugar species results in a dramatic improvement in selectivity. The formation constant for the glucose-borate complex is far smaller (~ 10^2) than the corresponding fructose complex (~10^4) (7). As a result, glucose does not give any detectable response under the experimental conditions employed here, making the determination of fructose entirely free from glucose interference. Changing these conditions to accomodate the lower stability of the glucose complex, e.g. by increasing borate ion concentration, would very likely permit determination of glucose also.

Although further work is required to clearly establish the optimum conditons necessary to separate all sugars, it would appear that reactions occur on the column to give suitable products for effective separation and detection. Further work along these lines is under way in this laboratory.

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